

Expression CMTM6 and PD-L1 and Their Significance in Cervical Squamous Cell Carcinomas

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Research

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Abstract

Background: This study aimed to investigate the expression levels of chemokine-like factor-like MARVEL transmembrane domain-containing family member 6 (CMTM6) and PD-L1 in cervical squamous cell carcinoma and their clinical significance.

Methods: We used immunohistochemistry to detect the expression of CMTM6 and PD-L1 in 41 cases of normal cervical squamous mucosa tissue and 76 cervical squamous cell carcinomas. Chi-square test was used to analyze the difference of CMTM6 between cervical squamous carcinoma and normal squamous mucosa.

Results: The results showed that the expression of CMTM6 and PD-L1 in cervical squamous carcinoma was significantly higher than that in normal squamous mucosa ($p < 0.05$). There is a positive correlation between the expression of CMTM6 and PD-L1 in cervical squamous carcinoma ($p < 0.05$). There is no correlation between CMTM6 and HPV infection in cervical squamous carcinoma. In addition, CMTM6 expression is positively correlated with lymph node metastasis and nerve invasion for cervical squamous carcinoma ($p < 0.05$). PD-L1 expression is positively correlated with the differentiation of cervical squamous carcinoma.

Conclusions: The expression of CMTM6 and PD-L1 is closely correlated in cervical squamous carcinoma, and may be used as a molecular target for cervical cancer in the future.

Background

Cervical cancer is one of the most common malignant tumors in women worldwide[1]. In China, the incidence of cervical cancer is only second to breast cancer, which seriously endangers women's health [2]. Approximately 90,000 women are diagnosed with cervical cancer every year in China, and the number of death due to cervical cancer is about 30,000 [3]. Early cervical cancer can be treated with good therapeutic effects with surgery, radiotherapy or chemotherapy, but for advanced cervical cancer or recurrent tumors that have metastasized, the treatment effect is much less favorable, and the 5-year survival rate is only 16.8%[4]. The inactivation of tumor suppressor genes and the activation of oncogenes play an important role in the pathogenesis of cervical cancer. Certain growth factors, cytokines and their receptors also play a role in the initiation and development of cervical cancer[5]. Therefore, searching for potential molecular targets for the diagnosis and treatment of cervical cancer has extremely important practical significance for early diagnosis of cervical cancer, improvement of quality of life and prognosis of cervical cancer patients.

Recently, PD-L1 has been reported to be an important molecular target for treatment in multiple types of cancers. One of the regulators that interact with PD-L1 is CMTM6. CMTM6 protein is a type III transmembrane protein with MARVEL-like domain, which is mainly located on the plasma membrane of the cell [9]. CMTM-6 binds to PD-L1 and maintains its expression on the cell surface, protecting PD-L1 from becoming lysosome-mediated degradation target [10, 11]. The deletion of CMTM6 in some tumors can reduce tumor-specific T cell activity and cause tumor immune escape, which indicates that CMTM6 gene may be a tumor suppressor gene in tumorigenesis [9]. However, it was found in cervical cancer cells that overexpression of CMTM6 protein did not affect the proliferation of cancer cells [12]. This suggests that CMTM6 may have different biological functions in different tumors.

The purpose of our research is to investigate the immunohistochemical expression of CMTM6 and PD-L1 and their significance in cervical squamous cell carcinomas.

Material And Methods

Selection of Patients

Tissue samples from cervical squamous cell carcinoma (n=76) and normal cervical tissue (n=41) were collected from surgical resection specimens in the Cangzhou People's Hospital(China). Among them, 44 cases have tumors accompanied with HPV infection. None of the patients underwent chemotherapy, radiotherapy or adjuvant treatment before surgery. Informed written consent was obtained from all participants and the study was approved The Ethical Committee of The Cangzhou People's Hospital approved the research protocol.

Immunohistochemical stain

Immunohistochemical stain (IHC) was performed on consecutive 4 µm-thick sections generated from cervical squamous cell carcinomas and normal cervical squamous mucosa. The unstained slides were deparaffinized with xylene three times, rehydrated with alcohol, and subjected to antigen retrieval by heating in target retrieval solution for 20 min in a microwave oven. The sections were quenched with 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Non-specific binding was prevented by adding 5% bovine serum albumin for 5 min. The sections were incubated with CMTM6 (Abcam, UK, 1:100) and PD-L1 (Roche sp263) for 2 hrs at room temperature, then incubated with anti-rabbit antibodies conjugated to HRP (Dako USA 1:200) for 1 hr. After each treatment all sections were washed three times with TBST and the binding sites were visualized with DAB. After counterstained with hematoxylin, the sections were dehydrated, cleared and mounted.

CMTM6 and PD-L1 proteins are located on the cell membrane. The stains were independently evaluated by two authors (MHY and SS). Any discrepancy was re-checked by both observers in a multiheaded microscope until a consensus was reached. The percentage of cells showing expression (positivity) was semi-quantitatively scored as : 0 =no cells stained; 1 = 1-50% cells stained; 2 = 50-74%; 3 ≥75%. The staining intensity score was graded as follows: 1 = weak; 2 = intermediate; and 3 = strong. The scores for CMTM6 positivity and staining intensity were multiplied to obtain a final score (H-score), which determines their expression as (- = 0; + = 1-2; ++ = 3-5; +++ = 6-9).

Statistical analysis

The SPSS 20.0 software program was used to analyze all data. The expression difference between cervical cancer and normal cervical tissue was analyzed using Chi-Square test. The correlation between the expression of CMTM6 and PD-L1 and clinicopathological parameters was analyzed by Chi-Square test and multivariate logistic regression analysis. McNemer test was used to analyze the correlation between CMTM6 and PD-L1 in cervical cancer. A p value less than 0.05 was considered statistically significant.

Results

CMTM6 and PD-L1 expression in cervical squamous cell carcinoma and normal cervical squamous mucosa

We included 76 cases of cervical cancer tissue and 41 cases of normal cervical tissue. As shown in Fig. 1, expression of CMTM6 and PD-L1 was seen on the cell membrane. The positive rates of CMTM6 in cervical cancer tissue and normal cervical tissue were 81.6% (62/76) and 0% (0/41), respectively ($p < 0.05$, Table 1). The positive rate of PD-L1 in cervical cancer tissue and normal cervical squamous mucosa was 78.9% (60/76) and 2.4% (1/41),

respectively ($p < 0.05$, Table 1). There is a positive correlation between the expression of CMTM6 and PD-L1 in cervical cancer tissues ($p < 0.05$, Table 2). No correlation was observed between CMTM6 and HPV infection in cervical cancer tissues ($p > 0.05$, Table 3).

Correlation of CMTM6 and PD-L1 expression with the clinicopathological parameters in cervical squamous cell carcinomas

We collected the clinicopathological data of 76 patients with cervical squamous cell carcinoma, including patient age, tumor size, tumor differentiation, clinical stage, lymphovascular invasion, perineural invasion, lymph node status, and distant metastasis. As shown in Table 4, CMTM6 expression is associated with lymph node metastasis and perineural invasion ($p < 0.05$). There was no correlation between CMTM6 expression and other clinicopathological features ($p > 0.05$). PD-L1 is only correlated to the degree of tumor differentiation ($p < 0.05$).

Discussion

Chemokines play an important role in tumor pathogenesis, autoimmune diseases and other processes, and provide new targets for treatment. CMTM family, consisting of 8 members (CMTM 1–8, previously known as human chemokine-like factor superfamily (CKLFSF))[6], plays an important role in tumorigenesis and development [7–8]. In this study, we investigated the immunohistochemical expression of one of the CMTM family members CMTM6 in a large series of cervical squamous cell carcinomas. We also investigated PD-L1, which is regulated by CMTM6 in these tumors. We found that CMTM6 and PD-L1 was expressed in 81.6% and 78.9% cervical squamous cell carcinomas, respectively. A positive correlation between the expression of CMTM6 and PD-L1 was also observed these tumors. CMTM6 expression was correlated with perineural invasion and lymph node status whereas PD-L1 expression was correlated with tumor differentiation.

CMTM6 is a widely expressed protein involved in epigenetic regulation, embryonic development and tumorigenesis. Studies had found that CMTM6 can act as a key regulator of PD-L1 in tumor cells, bind to PD-L1 and maintain its expression on the cell surface. CMTM6 coexists with PD-L1 in cells, CMTM6 can prevent PD-L1 from becoming a target of lysosome-mediated degradation. CMTM6 gene knockout only causes the reduction of PD-L1 protein on the cell surface, but does not reduce the level of PD-L1 mRNA, so CMTM6 can regulate the level of PD-L1 at the protein level[10, 11]. The reduction of PD-L1 expression level significantly reduces the effect of inhibiting tumor-specific T cell activity[14]. CMTM6 shows obvious specificity in preventing PD-L1 from being degraded, and does not express antigen through MHC class I molecules. This suggests that CMTM6 can be used as a potential therapeutic target and enhance anti-tumor immunity to a certain extent[15].

In non-small cell lung cancer and oral squamous cell carcinoma, a positive correlation between CMTM6 protein level and PD-L1 protein level was observed [16, 17]. Our research results show there was a positive correlation between the expression of CMTM6 and PD-L1 in cervical squamous cell carcinoma. In hepatocellular carcinoma, the level of CMTM6 protein is significantly lower than that of adjacent tissues. In addition, the level of CMTM6 protein is related to pathological grade, tumor metastasis and AFP level in hepatocellular carcinoma [18]. In breast carcinoma, the expression level of CMTM6 is significantly higher than that of adjacent normal breast tissue, and is related to pathological stage and positive HER2 expression [19]. However, we found that the expression of CMTM6 in cervical cancer was significantly higher than that in normal cervical squamous mucosa. In our cervical squamous carcinomas, CMTM6 expression is associated with lymph node metastasis and nerve invasion. There was no relationship between CMTM6 expression and other clinicopathological features of cervical cancer patients. PD-L1 is

only related to the degree of tumor differentiation. The above results indicate that CMTM6 has different expression levels in different tumors and different prognostic significance.

Conclusions

In summary, upregulated CMTM6 expression was found to be associated with lymph node metastasis and nerve invasion in cervical squamous cell carcinomas. PD-L1 was found to be associated with differentiation in these tumors. CMTM6 and PD-L1 may be potential molecular targets for cervical squamous cell carcinoma. Whether CMTM6 and PD-L1 can be used as a marker for cervical cancer invasion, metastasis and prognosis still needs a large number of samples to verify. The possible role of CMTM6 and PD-L1 in pathogenesis in cervical squamous cell carcinoma needs further verification.

Declarations

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Authors' contributions

MHY performed all experiments and made substantial contributions to analysis

and drafting the manuscript. SS collected the clinical data; MZH contributed to sample collection. ZZG and LHG designed the study and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table.1 CMTM6 and PD-L1 expression in Normal tissue and Cervical cancer tissue

Groups	CMTM6 expression				PD-L1 expression		
	n	-	+	PR(%)	-	+	PR(%)
Normal	41	41	0	0	40	1	2.4
Cancer	76	14	62	81.6[□]	16	60	78.9[□]

PR=positive rate, [□]p < 0.05.

Table.2 Correlation analysis of CMTM6 and PD-L1 in cervical cancer

CMTM6	PD-L1		Total	p value
	Positive	Negative		
Positive	52	10	62	0.027
Negative	8	6	14	
Total	60	16	76	

Table.3 Correlation analysis of CMTM6 and HPV in cervical cancer

CMTM6	HPV		Total	p value
	Positive	Negative		
Positive	26	8	34	0.678
Negative	7	3	10	
Total	33	11	44	

Table.4 Relationship between expression of CMTM6 and PD-L1 protein and Clinicopathological features of Cervical cancer

Clinicopathological features	expression												
	CMTM6							PDL-1					
	n	-	+	++	+++	PR(%)	<i>P</i> value	-	+	++	+++	PR(%)	<i>P</i> value
Age													
≤50	41	7	12	17	5	82.9	0.731	6	15	14	6	85.4	0.871
≥50	35	7	11	12	5	80.0		8	10	5	12	77.1	
Size													
≤3	34	5	13	13	3	85.3	0.164	6	13	9	6	82.4	0.379
≥3	42	9	10	16	7	78.6		8	12	10	12	81.0	
Lymph node metastasis													
-	61	11	19	26	5	82.0	0.049	11	19	16	15	82.0	0.842
+	15	3	4	3	5	80.0		3	6	3	3	80.0	
Distant metastasis													
-	75	14	22	29	10	81.3	0.627	14	24	19	18	81.3	0.613
+	1	0	1	0	0	100.0		0	1	0	0	100.0	
Clinical stage													
I	46	10	17	13	6	78.3	0.372	10	17	7	12	78.3	0.234
II-III	30	4	6	16	4	86.7		4	8	12	6	86.7	
Vascular infiltration													
-	35	7	12	13	3	80.0	0.572	6	12	9	8	82.9	0.552
+	41	7	11	16	7	82.9		8	13	10	10	80.5	
Nerve invasion													
-	66	14	21	22	9	78.8	0.010	13	19	17	15	80.3	0.655
+	10	0	2	7	1	100.0		1	4	2	3	90.0	
Differentiation													
Well-differentiated	4	1	1	2	0	75.0	0.770	0	3	1	0	100.0	0.030
Moderately-differentiated	59	13	19	20	7	78.0		12	20	14	13	79.7	
Poorly-differentiated	13	0	3	7	3	100.0		2	2	4	5	84.6	

Figures

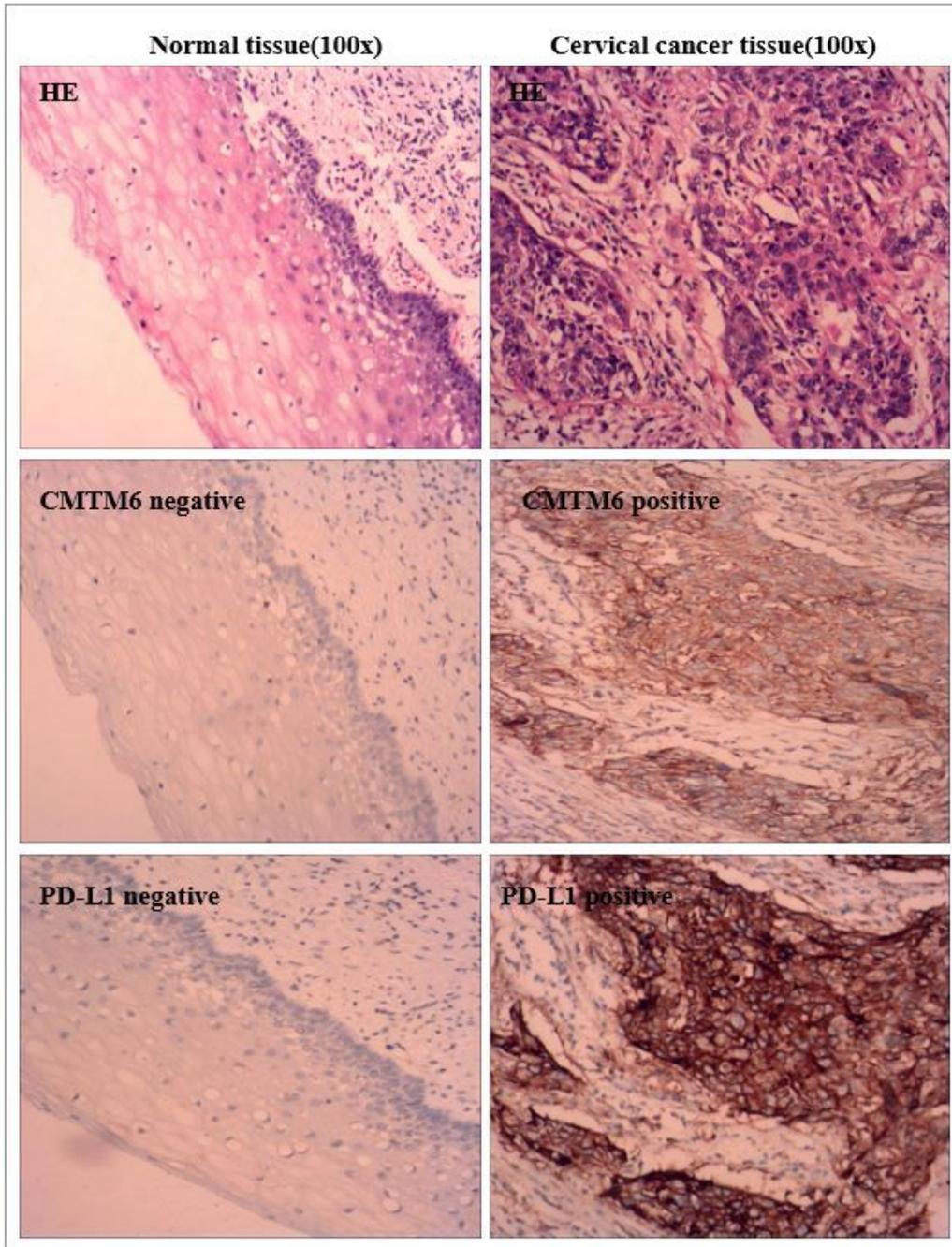


Figure 1

Expression of CMTM6 and PD-L1 in Normal tissue and Cervical cancer tissue. Representative positive and negative expression of CMTM6 and PD-L1 were detected by immunohistochemistry (magnification at $\times 100$).